Innovative Food Science and Emerging Technologies 9 (2008) 418–422

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Comparison of hydrostatic and hydrodynamic pressure to inactivate foodborne viruses

Manan Sharma [a,*], Adrienne E.H. Shearer [b], Dallas G. Hoover [b], Martha N. Liu [a], Morse B. Solomon [a], Kalmia E. Kniel [b]

[a] Food Safety Laboratory, Building 201, USDA-ARS, ANRI, 10300 Baltimore Ave. Beltsville, MD 20705, USA
[b] Department of Animal and Food Sciences, University of Delaware, 531 South College Ave, Newark, DE, 19716, USA

ARTICLE INFO

Article history:
Received 29 December 2007
Accepted 7 May 2008

ABSTRACT

The effect of high hydrostatic pressure (HPP) and hydrodynamic pressure (HDP), in combination with chemical treatments, was evaluated for inactivation of foodborne viruses and non-pathogenic surrogates in a pork sausage product. Sausages were immersed in distilled water, 100-ppm EDTA, or 2% lactoferrin, and then inoculated with feline calicivirus (FCV), hepatitis A virus (HAV) or bacteriophage (MS2, phiX174, or T_4). Each piece was packaged individually and subjected to pressure by either HDP, HPP (500 MPa, 5 min, 4 °C), or control (no pressure). On sausages immersed in water, HPP and HDP significantly ($P<0.05$) reduced titers of FCV by 2.89 and 2.70 $\log_{10}$ $TCID_{50}$/ml, and HAV by $\log_{10}$ 3.23 and 1.10, respectively, when compared to non-pressure-treated controls. Titers of T_4 (1.48 and 1.10 $\log_{10}$ PFU/g) and MS2 (1.46 and 0.96 $\log_{10}$ PFU/g) were also significantly reduced by HPP and HDP treatments, respectively, in combination with water. Inoculation of viruses and bacteriophage on a meat product may have protected viruses from complete inactivation by pressure treatments.

Industrial relevance: This is the first study to directly compare hydrostatic and hydrodynamic pressure technologies to inactivate microorganisms. This is also the first study to examine the inactivation of viruses and bacteriophages by pressure technology in a deli meat product. This study shows that viruses attached to meat surfaces may be protected from complete inactivation by hydrostatic and hydrodynamic pressure treatments, and these findings require more investigation into the survival of viruses in deli meat products.

Published by Elsevier Ltd.

1. Introduction

Viral foodborne infections account for 80% of cases of foodborne illness caused by known etiological agents in the United States each year (Mead et al., 1999). These cases may be attributed to a variety of enteric human pathogens including norovirus, hepatitis A virus, adenovirus, rotavirus, and Aichi virus. Noroviruses are estimated to cause a large percentage (66.6%) of the total number of cases of foodborne illness and are by far the leading cause of viral foodborne illness in the United States. Cases of viral gastroenteritis are thought be underreported because of the difficulty of detection and lack of reporting by patients. Noroviruses have proven difficult to work with because of the lack of culturability in laboratory cell lines, and the majority of researchers have used other non-enveloped viruses or bacteriophage, like feline calicivirus (FCV) or MS-2 (a coliphage), as surrogates that can be cultured in the laboratory (Koopmans and Duizer, 2004).

Hepatitis A (HAV) has been implicated in an increasing number of foodborne outbreaks. The majority of these cases are transmitted by contaminated food handlers; however, in 2003, the largest outbreak in the U.S. of HAV was associated with the consumption of contaminated green onions imported from Mexico when nearly 1000 individuals became ill and 4 died (Wheeler et al., 2005). The large number of people associated with virally transmitted foodborne illness is a call for an improved understanding of the interactions of viruses with different food matrices.

This study assesses the use of two non-thermal treatments to inactivate viruses on a meat sausage product. Non-thermal processes are of interest because they induce less sensory changes to a product than thermal processing while providing microbial inactivation. The utilization of non-thermal processing technologies as an alternative to the addition of antimicrobial additives to deli meats and other ready-to-eat meat products is gaining popularity with consumers and industry alike (Clark, 2006). High hydrostatic pressure processing (HPP) is a non-thermal technology that is being used to inactivate foodborne viruses while maintaining the fresh-like attributes of foods. HPP is currently used in the processing of deli meats, salsa, fruit juices, and shellfish. While ready-to-eat meat products are not traditionally considered to be at risk for natural contamination by viruses, post-processing contamination by human handlers may occur. Studies have shown that norovirus inoculated on a variety of surfaces persists for up to 7 days (D'Souza et al., 2006). Noroviruses may also have the potential for zoonotic transmission. Human norovirus strains (genotype GII.4)

* Corresponding author.
E-mail address: manan.sharma@ars.usda.gov (M. Sharma).

1466-8564/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.ifset.2008.05.001

were detected in animal fecal samples and in a retail meat samples (Mattison, Shukla et al., 2007). Other work has shown that higher levels of FCV, a norovirus surrogate, were recovered from deli meat than from lettuce (Mattison, Karthikeyan et al., 2007), indicating that viruses could potentially survive on the surface of foods. Work with other human enteric viruses including hepatitis A and bacteriophage surrogates is essential to understand mechanisms to reduce illness from post-processing contamination.

The lethality of pressure treatment is affected by the food matrix, including salt and fat composition, and by the time and temperature parameters of the process. A 7-$\log_{10}$ $TCID_{50}$ (tissue culture infectious dose)/ml decrease in infectivity was observed for FCV in liquid suspension when 275 MegaPascals (MPa) of pressure was applied for 5 min (Kingsley, Hoover, Papafragkou, & Richards, 2002). Other investigators have shown that FCV inactivation at 200 MPa was significantly enhanced at extremely cold (−10 °C) or high (50 °C) temperatures when compared to treatment at 20 °C (Chen, Hoover, & Kingsley, 2005). Hepatitis A virus (HAV) was inactivated by more than 7 $\log_{10}$ $TCID_{50}$/ml at 450 MPa for 5 min, but the presence of seawater increased the pressure resistance of the virus (Kingsley et al., 2002). The addition of calcium (Ca^{2+}) ions to suspensions of *Escherichia coli* also increases the pressure resistance of these bacterial cells (Hauben, Bernaerts, & Michiels, 1998).

Hydrodynamic pressure processing (HDP) is a non-thermal process that involves the underwater detonation of an explosive in a container creating a shockwave pressure front between 70 and100 MPa that passes through water and meat. The compression of this water (adiabatic) causes a temperature increase of 2.5 °C per 100 MPa. In meat, HDP has been shown to improve tenderness by disrupting myofibrillar structure in the muscle (Solomon, Liu, Patel, Bowker & Sharma, 2006). Although the microbiocidal mechanism of HDP remains largely uncharacterized, it has been postulated that a shock wave–cavitation interaction contributes to this effect. The shockwave creates a tensile stress, which results in the formation of microcavities. Cavitation, the collapsing of microcavities, creates localized high pressure and high temperature gradients that may inactivate bacterial cells (Alvarez, Loske, Castano-Tostado, & Prieto, 2004).

HDP has had a variable effect on the inactivation of foodborne pathogens. Pork loins inoculated with the parasite, *Trichinella spiralis*, and subsequently treated with HDP had fewer larvae per gram (LPG) than control (non-HDP treated) loins (Gamble, Solomon, & Long, 1998), but no HDP-treated samples were completely free of *T. spiralis*. Studies with HDP and bacterial foodborne pathogens have been less conclusive. Total bacterial counts recovered from HDP-treated ground beef and beef stew pieces were reduced by 1–2.5 log CFU/g compared to controls not treated with HDP (Williams-Campbell and Solomon, 2002). In this same study, the shelf-life of HDP-treated ground beef was extended by seven days and had 4–5 log CFU/g less total counts compared to non-HDP treated ground beef.

The objective of this study was to compare inactivation of viral surrogates (bacteriophage) and foodborne viruses by hydrodynamic and hydrostatic pressure inactivation in a contaminated deli meat product and to assess the impact of the food matrix and chelating agents may have in enhancing or diminishing the effectiveness of pressure on foodborne viruses.

2. Materials and methods

2.1. Sausage preparation

Edible lean beef trim and pork fat trimmings were obtained and frozen from animals used in other studies from the USDA Food Safety Lab (FSL, Beltsville, MD). Meat was chopped in a bowl cutter (Talsa, Holy Sales & Service, Inc., Elkridge, MD) with 16.3% ice and 2.1% salt (Alberger Fine Flake, Cargill, Minneapolis, MN) to produce the emulsion batter. The batter was stuffed into collagen casings (17D39, Superfry Plus, Nitta Casings, Somerville, NJ) with a vacuum stuffer (VF-50, Handtman, Buffalo Grove, IL). Product was cooked in a smokehouse (700HP, Alkar-RapidPak, Lodi, WI) using the following schedule: 20 min at 65.5 °C dry bulb temperature, 48.9 °C wet bulb temperature and 40% relative humidity; 10 min at 76.7 °C dry bulb temperature, 60.0 °C wet bulb temperature and 44% relative humidity; 76.7 °C steam cooked to 71.1 °C product core temperature followed by 15 min of cold water shower. After cooking, the sausage underwent further cooling and storage at 2 °C for 24 h. The finished diameter of the links ranged from 14 to 16 mm. Links were separated from each other and vacuum packaged in 3 mil high performance vacuum-packaging bags (Model 030044, Koch, Kansas City, MO). Packaged samples were frozen at −20 °C until testing. Samples were thawed at 4 °C overnight before pressure treatments were performed.

2.2. Treatment and inoculation of sausage

After samples had thawed, sausage links were cut with a sterile knife in approximately 2.5 cm pieces, then weighed and placed in a sterile container with either 100 ppm EDTA (Sigma Co, St. Louis, MO), 2% lactoferrin (DMV International, Delhi, NY), or sterile water for 5 min. Sausage pieces were removed and placed on a sterile rack to dry for 10 min. A total of 50 μl of virus or bacteriophage was deposited and spread with a pipet tip on the surface of the sausage and allowed to dry for 30 min. Coliphages tested were MS2 (1×10^{10} PFU/ml, host *E. coli* ATCC 15597B-1), phix174 (3.64×10^{10} PFU/m, host *E. coli* ATCC 11303B), and T_4 (5.78×10^{8} PFU/ml, host *E. coli* ATCC 13706B). Viruses examined were feline calicivirus (FCV) and hepatitis A ($1\times10^{8-9}$ $TCID_{50}$/ml). Inoculated sausages were allowed to dry for 15 min under a sterile hood before packaging for pressure treatment.

2.3. Hydrodynamic pressure treatment

After drying, sausages were placed into nylon vacuum pouches (3 Mil standard barrier; Cryovac Sealed Air Corporations, Duncan, SC) and vacuum packaged (model LV 10 g ; Hollymatic Corporations, Countryside, IL). All packages (HDP-treated and control) were placed in boneguard bags (Cryovac), sealed at one end, and vacuum packaged. Each package contained nine inoculated pieces of sausage. Boneguard bags were then heat shrunk in 88 °C water for 3 s to remove air pockets in bags.

Packaged samples were placed in a 98-l plastic explosive container (PEC) (Rubbermaid, Fairlawn, OH) containing a flat steel reflector plate and filled with water and ice to create a temperature between 1.5 and 13.2 °C. Packaged samples were fastened to the reflector plate and then placed at the bottom of the PEC. A binary explosive (100 g, cylindrically shaped), based on a proprietary mixture of ammonium nitrate and solvents, was immersed in the water 22.5 cm above the package and the explosive was detonated. After the detonation, the packaged was unfastened from the reflector plate, sanitized, opened, and the sausages were removed with sterile forceps and placed into 10 ml of 3% beef extract broth (Becton Dickinson, Sparks, MD).

2.4. Hydrostatic pressure treatment

Inoculated sausages were aseptically placed into sterile polypropylene pouches (VWR International, West Chester, PA). The pouches were heat-sealed and then sealed in a secondary pouch. Pressure treatments were applied in a laboratory-scale Avure PT-1 hydrostatic pressure unit (Avure Technologies Inc., Kent, WA). Water was the transmitting medium. The come-up rate was approximately 20 MPa/s and the pressure release time was <4 s. Samples were treated under the following conditions: 500 MPa, 5-min hold time (exclusive of come-up time of approximately 30 s with depressurization occurring within 2–3 s), and surrounding water bath temperature of 4 °C. The temperature of the medium of decreased from 12.7 °C to 4.3 °C during the five minute treatment time. Adiabatic heat of the water was measured to be 2.5 °C for every 100 MPa increase in pressure.

2.5. Enumeration

Pressure-treated sausage inoculated with bacteriophage or viruses were placed in a sterile 50-ml conical tube containing 10 ml of sterile 3% beef extract (Becton Dickinson, Sparks, MD), adjusted to pH 9.4, and then vortexed for 3 min. After vortexing, 100 μl of bacteriophage in 3% beef extract were serially diluted into 900 μl sterile deionized water, mixed with the appropriate host *E. coli* strain and 0.75% Luria Bertrani (LB) (Becton Dickinson) soft agar, and overlaid on LB agar plates. Plates were then incubated at 37 °C for 24 h before plaques were counted.

Viruses in beef extract were brought to pH 7.4 by the addition of 1N HCl and then serially diluted and assayed in 96-well plates confluent with host cells. Virus infectivity was determined by reading the cytopathic effect 3–5 days post-inoculation (dpi) for FCV and 14 dpi for HAV. FCV, ATCC VR-651, was assayed on Crandell Reese feline kidney cells (CRFK), ATCC CCL-94, in Minimal Essential Medium (MEM, Mediatech, Herndon VA) and HAV,ATCC VR-1402 was assayed on fetal rhesus monkey kidney cells (FRhK-4) ATCC CRL-1688 in Dulbecco's modified Eagle medium (DMEM, Mediatech). Growth medium was supplemented with 2% fetal bovine serum (FBS, Mediatech), penicillin/streptomycin (Mediatech), amphotericin B (Mediatech), sodium bicarbonate (Mediatech), and sodium pyruvate (Mediatech). Virus stocks were propagated and stored in medium with 2% FBS at −80 °C until use. Viral titers were determined using $TCID_{50}$ and calculated using the Reed Meunch method.

2.6. Statistical analysis

Three replicates of each experiment were performed. Viral and bacteriophage titers were statistically analyzed (SAS version, 9.1, Cary, NC) using analysis of variance and least significant difference mean separation tests ($P<0.05$).

3. Results

3.1. Inactivation of FCV and HAV on sausages

Table 1 displays recovery of feline calicivirus and hepatitis A from sausages immersed in 2% lactoferrin, 100 ppm EDTA or water (control) and then subsequently pressure-treated. Samples inoculated with FCV and HPP- or HDP-treated, regardless of immersion treatment, had lower titers ($P<0.05$) than the controls (no pressure treatment). Similarly, HAV titers recovered from HPP-treated sausages, regardless of chemical treatment, were also significantly lower than those recovered from controls. HAV titers from sausages immersed in water and treated with HDP were significantly greater than titers from HPP-treated sausages, but significantly lower than those recovered from sausages that were not pressure-treated. There were no significant differences in the titers of HDP- and control-treated sausages inoculated with HAV when immersed in either EDTA or lactoferrin. There were no differences ($P>0.05$) in titers of viruses from sausages inoculated with HAV and immersed in 100 ppm EDTA, or 2% lactoferrin, compared to those immersed in water when receiving the same pressure treatments, indicating that these chemical treatment did not affect virus inactivation on sausages.

The largest reduction of FCV and HAV titers on sausage were 2.89 and 3.23 log $TCID_{50}$/ml, respectively, when treated with a combination of water and HPP and compared to control. The combination of water and HDP treatment resulted in a reduction of 2.71 log $TCID_{50}$/ml of FCV and 1.10 log $TCID_{50}$/ml reduction of HAV on sausages, when compared to non-pressure-treated controls.

Table 1
Titers (log_{10} $TCID_{50}$/ml) of feline calicivurs (FCV) and Hepatitis A (HAV) recovered from sausages immersed in water, 100 mM EDTA, 2% lactoferrin and treated with hydrostatic (HPP) or hydrodynamic (HDP) pressure or control (no pressure)

Virus	Chemical treatment	Pressure treatment ($TCID_{50}$)/ml[1]		
		HPP	HDP	Control
FCV	Water	a 4.00 b[2]	a 4.19 b	a 6.89 a
	EDTA	a 3.79 b	a 3.72 b	a 6.15 a
	Lactoferrin	a 4.28 b	a 4.37 b	a 6.23 a
HAV	Water	a 3.78 c	a 5.91 b	a 7.01 a
	EDTA	a 3.79 b	a 5.59 a	a 5.76 a
	Lactoferrin	a 3.94 b	a 5.54 a	a 6.04 a

[1]Within the virus and pressure treatment, different letters preceding mean values indicate significant ($P<0.05$) differences caused by chemical treatments.
[2]Within virus and chemical treatment, different letters following mean values indicate significant ($P<0.05$) differences caused by pressure treatments.

Table 2
Titers (log_{10} PFU/g) of coliphages recovered from sausages immersed in water, 100 mM EDTA, or 2% lactoferrin and treated with hydrostatic (HPP) or hydrodynamic (HDP) pressure or control (no pressure)

Coliphage	Chemical treatment	Pressure treatment (log PFU/g)[1]		
		HPP	HDP	Control
T_4	Water	ab 4.81 b[2]	a 5.19 b	a 6.29 a
	EDTA	b 4.66 b	a 5.28 b	a 6.17 a
	Lactoferrin	a 5.16 b	a 5.30 b	a 6.26 a
MS2	Water	a 5.34 b	ab 5.85 b	a 6.81 a
	EDTA	a 5.39 b	b 5.21 b	a 6.71 a
	Lactoferrin	a 5.69 a	a 6.22 a	a 6.86 a
phiX174	Water	b 4.13 b	a 5.42 ab	a 5.69 a
	EDTA	ab 4.23 b	a 4.96 ab	a 5.68 a
	Lactoferrin	a 4.45 b	a 5.33 ab	a 5.83 a

[1]Within the bacteriophage and pressure treatment, different letters preceding mean values indicate significant ($P<0.05$) differences caused by chemical treatments.
[2]Within bacteriophage and chemical treatment, different letters following mean values indicate significant ($P<0.05$) differences caused by pressure treatments.

3.2. Inactivation of bacteriophage on sausages

Sausages inoculated with T_4 and HPP or HDP-treated had significantly lower titers than the controls, regardless of chemical treatment (Table 2). Titers from sausages inoculated with MS2, immersed in EDTA or water, and then HPP- or HDP-treated were significantly lower than non-pressure-treated control samples. Regardless of chemical treatments, significantly lower titers from sausages inoculated with phiX174 and HPP-treated were recovered than from those not receiving pressure treatments. No statistical differences in titers were observed for sausages inoculated with phiX174 and HDP- or control-treated. Sausages inoculated with T_4 and HPP-treated has significantly lower titers when immersed in EDTA than in lactoferrin (Table 2). Titers of MS2 from inoculated sausages treated by HDP were also significantly lower when immersed in EDTA than in lactoferrin. Significantly lower recoveries of phiX174 were observed when sausages were HPP-treated and immersed in water rather than lactoferrin. No significant differences in titers of MS2 on sausages immersed in lactoferrin and HPP-, HDP- or control-treated were observed.

Reductions of coliphage titers on sausages resulting from a combination of HPP and water treatment when compared to non-pressure-treated controls and ranged from 1.48–1.56 log PFU/g. The combination of HDP and water reduced titers of T_4 on sausage by 1.10 log PFU/g compared to control. The combination of HDP and EDTA reduced titers of MS2 by 1.5 log PFU/g compared to control.

4. Discussion

To our knowledge this is the first study that has compared microbial inactivation in a food product by both high hydrostatic pressure (HPP)

and hydrodynamic pressure processing (HDP). Microbial inactivation by HDP treatment has resulted in varied results in previous studies. Patel, Williams-Campbell, Liu, and Solomon (2005) reported minimal reductions (0.3 log CFU/g) in populations of *E. coli* O157:H7 on intact beef muscle treated by HDP compared to untreated controls. Similarly, populations of *Salmonella* on HDP-treated (100 g explosive) minced chicken were only reduced by 0.3 log CFU/g compared to non-HDP treated controls (Patel, Bhagwat, Sanglay, & Solomon, 2005). However, HDP treatments significantly ($P<0.05$) reduced bacterial populations of *Listeria monocytogenes* on beef frankfurters, but reductions were less than 1 log CFU/g over non-treated (control) samples (Patel, Sanglay, Sharma, & Solomon, 2007). Total aerobic bacterial counts in ground beef treated with HDP was reduced by 1–2.5 log CFU/g compared to untreated samples (Williams-Campbell & Solomon, 2002).

Variability in inactivation by HDP, previously observed in other studies examining bacteria and parasites, was also observed in our study on viruses and phages. Titers of FCV and T_4 were consistently reduced by HDP regardless of chemical treatment. The combination of immersion in water and HDP treatment significantly inactivated four (FCV, HAV, T_4, and MS2) of the five viruses when compared to controls, while the combination of EDTA and HDP significantly inactivated two (FCV and T_4) of the five viruses when compared to controls. Titers of phiX174 were not significantly reduced by HDP treatment in combination with chemical treatment. Previous work attributed the hydrostatic pressure sensitivity of T_4 to relatively complex sheathed and contractible tail structure compared to other phage (MS2, phiX174) (Guan et al., 2006). These same elements may also make T_4 more sensitive to HDP as well.

Our results showing similar levels of inactivation of FCV and HAV by HPP and HDP were surprising. Inactivation of viruses and phages on the surfaces of sausages by pressure treatments may have been reduced by attachment to the meat surface. Titers of murine norovirus-1 (MNV-1) in DMEM with 10% FBS were reduced by 6.85 $\log_{10}$ PFU when treated at 450 MPa for 5 min at 20 °C (Kingsley, Holliman, Calci, Chen, & Flick, 2007). A log reduction of 2.61 PFU of QB phage, a potential surrogate for HAV, was achieved in oyster meat after HPP treatment at 550 MPa for 10 min at 20 °C (Smiddy, Kelly, Patterson, & Hill, 2006). These levels were similar to our findings, with titers of FCV and HAV reduced titers by 2.89 and 3.23 log $TCID_{50}$/ml on sausages, respectively, when treated by HPP. QB phage reductions ranging from 5.51–5.95 log PFU in oyster tissue were achieved at 700 MPa for 10 min at 20 °C (Smiddy et al., 2006). Other studies have shown that titers were 2.76 log PFU in HAV-inoculated oysters treated with 400 MPa hydrostatic pressure for 1 min at an initial temperature of 9 °C; oysters receiving control treatment (no pressure) had HAV titers of 5.82 log PFU (Calci, Meade, Tezloff, & Kingsley, 2005). HAV titers in strawberry puree and sliced green onions were reduced by 4.32 and 4.75 log PFU, respectively (Kingsley, Guan, & Hoover, 2005) when compared to non-pressure-treated sausages. HAV was more pressure sensitive in strawberry puree than in sliced green onions.

Our study agrees with previous work stating that enteric viruses and phages are less sensitive to hydrostatic pressure in or on a food than in liquid suspension. HPP treatment at 275 MPa inactivated 7-log $TCID_{50}$ of FCV in Dulbeccco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS)when treated for 5 min at 20 °C (Kingsley et al., 2002), a much higher reduction than assays in our study at 500 MPa, which resulted in reductions of 2.9 log $TCID_{50}$. Modifications in temperature have been effective in reducing FCV titers with HPP — 200 MPa applied at −10 °C, 20 °C and 50 °C reduced titers of FCV in DMEM by 5, 0.3, and 4 PFU/ml, respectively (Chen et al., 2005). These authors postulated that hydration of hydrophobic amino acid residues of capsid proteins may disrupt tertiary and quaternary structure to inactivate viruses. HAV inactivation studies have shown that RNA is not released from the capsid after HPP treatment, indicating that the capsid structure of HAV remains intact but these proteins may have become denatured and may affect virion uncoating and attachment mechanisms (Kingsley et al., 2002). Attachment to meat surfaces may protect viral particles from pressure inactivation and protein denaturation observed when viruses in liquid suspension are pressure-treated.

Some workers have postulated that the higher the ionic strength of the medium or food, the more hydrostatic pressure resistance is afforded to HAV (Kingsley et al., 2005). FCV infectivity (log $TCID_{50}$) was higher on mussels (2.84) and oysters (2.17) than in seawater (0.58) and in culture (1.58) when pressure-treated at 250 MPa for 5 min at 20 °C (Murchie, Kelly, Wiley, Adair, & Patterson, 2007). HAV pressure resistance was enhanced in seawater with 27.4-ppm final salt concentration compared to isotonic tissue culture medium (Cascarino, 2007; Kingsley et al., 2002). In our study, treatments that served to decrease the ionic strength of the food did not affect the recovery of viruses or phages from sausages. The salt (NaCl) concentration of the sausage was 2.09% in sausage formulations. The lack of differences in recovered titers from sausages inoculated with FCV and HAV treated with EDTA, lactoferrin and water and pressure indicate that these chelating treatments did not increase the inactivation of viruses on sausages. The reason that chelating treatments slightly increased titers of recovered bacteriophage on sausage on some HPP and HDP-treated sausages is unclear. It is possible that the salt concentration in the original formulation was not sufficient to elicit change by chelating treatment. It is also possible that concentration of chelating treatments (100 ppm EDTA, 2% lactoferrin) were not high enough to affect recoverability of viruses and phages. Lactoferrin is approved for use at 2% in uncooked beef (Taylor, Brock, Kruger, Berner, & Murphy, 2004) and blocks attachment of bacterial pathogens to meat surfaces (Naidu, 2002). EDTA is approved for use in various foods at levels between 36 and 500 ppm in the U.S. (Anonymous, 2006) and was effective in binding calcium ions and reducing *E. coli* populations when treated by HPP (Hauben et al., 1998). Neither chemical, in combination with pressure treatment, was effective in reducing viral or coliphage titers on sausages.

Previous work by other authors determined that phiX174 would be a poor choice as a surrogate for HAV because its overall resistance to HPP inactivation at a range of pressures (350–600 MPa) in liquid suspension (Guan et al., 2006). Results from our study were in agreement with previous findings. HAV titers on sausage were reduced by >1.9 $\log_{10}$ $TCID_{50}$/ml for all HPP treatments; however, the largest reduction of phiX174 titers on sausage by HPP was <1.5 $\log_{10}$ PFU/g, indicating that greater pressure resistance was displayed by phiX174 than HAV. Titers of phiX174 on sausages immersed in EDTA or lactoferrin and treated with HDP did behave similarly to HAV.

MS2 and FCV, surrogates that have been used previously for norovirus, behaved differently from each other on pressure-treated sausages in our study. Reduction of FCV titers on sausage by HPP were >1.95 $\log_{10}$ $TCID_{50}$/ml greater than controls, compared to those of MS2 on sausage (< 1.17 $\log_{10}$ PFU/g), indicating that MS2 showed more pressure resistance than FCV on sausage. Titers of FCV were reduced >1.86 $\log_{10}$ $TCID_{50}$/ml when HDP-treated, while titers of MS2 were reduced by <1.5 $\log_{10}$ PFU/g). These differences in pressure sensitivities may indicate that FCV may be more appropriate for norovirus as a pressure surrogate.

This study was the first to directly compare the effects of HDP and HPP on inactivation viruses on meat product. Pressure treatments were effective in achieving some inactivation of viruses in a deli meat product, but viruses were protected from higher levels of inactivation due to attachments to meat surfaces. More work is needed to evaluate the survival and inactivation of foodborne viruses in deli meat products, and to identify appropriate surrogates for viruses so that appropriate non-thermal pressure technology may be developed to simultaneously preserve quality attributes and kill viral pathogens on deli meats.

Acknowledgments

The authors would like to thank Cheryl Mudd, Janice Callahan, Janet Eastridge, and Ernie Paroczay at USDA-ARS, and Dongsheng Guan at General Mills, Inc. for their technical assistance.

References

Alvarez, U. M., Loske, A. M., Castano-Tostado, E., & Prieto, P. E. (2004). Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* by underwater shock waves. *Innovative Food Science and Emerging Technologies*, *5*, 459–463.

Anonymous (2006). U.S. Food and Drug Administration. *Title 21, Part 172 — Food Additives Permitted for Direct Addition to Food for Human Consumption, Section 172.135. Revised April 1, 2006*.

Calci, K. R., Meade, G. K., Tezloff, R. C., & Kingsley, D. H. (2005). High-pressure inactivation of hepatitis A virus within oysters. *Applied and Environmental Microbiology*, *71*(1), 339–343.

Cascarino, J. (2007). Inactivation of viruses by high hydrostatic pressure in ready-to-eat food products. Unpublished master's thesis, University of Delaware, Newark, DE.

Chen, H., Hoover, D. G., & Kingsley, D. H. (2005). Temperature and treatment time influence high hydrostatic pressure inactivation of feline calicivirus, a norovirus surrogate. *Journal of Food Protection*, *68*(11), 2389–2394.

Clark, J. P. (2006). High-pressure processing research continues. *Food Technology*, *60*(2), 63–65.

D' Souza, D. H., Sair, A., Williams, K., Papafragkou, E., Jean, J., Moore, C., et al. (2006). Persistence of caliciviruses on environmental surfaces and their transfer to food. *International Journal of Food Microbiology*, *108*, 84–92.

Gamble, H., Solomon, M. B., & Long, J. (1998). Effects of hydrodynamic pressure on the viability of *Trichinella spiralis* in pork. *Journal of Food Protection*, *61*(3), 637–639.

Guan, D., Kniel, K., Calci, K. R., Hicks, D. T., Pivarnik, L. F., & Hoover, D. G. (2006). Response of four types of coliphage to high hydrostatic pressure. *Food Microbiology*, *23*, 546–551.

Hauben, K. J. A., Bernaerts, K. M., & Michiels, C. W. (1998). Protective effect of calcium on inactivation of *Escherichia coli* by high hydrostatic pressure. *Journal of Applied Microbiology*, *85*, 678–684.

Kingsley, D. H., Guan, D., & Hoover, D. G. (2005). Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. *Journal of Food Protection*, *68*(8), 1748–1751.

Kingsley, D. H., Holliman, D. R., Calci, K. R., Chen, H., & Flick, G. J. (2007). Inactivation of a norovirus by high-pressure processing. *Applied and Environmental Microbiology*, *73*(2), 581–585.

Kingsley, D. H., Hoover, D. G., Papafragkou, E., & Richards, G. P. (2002). Inactivation of hepatitis A virus and a calicivirus by high hydrostatic pressure. *Journal of Food Protection*, *65*(10), 1605–1609.

Koopmans, M., & Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, *90*, 23–41.

Mattison, K., Karthikeyan, K., Abere, M., Malik, N., Sattar, S. A., Farber, J. M., et al. (2007). Survival of calicivirus in foods and on surfaces: experiments with feline calicivirus as a surrogate for norovirus. *Journal of Food Protection*, *70*(2), 500–503.

Mattison, K., Shukla, A., Cook, A., Pollari, F., Friendship, R., Kelton, D., et al. (2007). Human noroviruses in swine and cattle. *Emerging Infectious Diseases*, *13*(8), 1184–1188.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., et al. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, *5*, 607–625.

Murchie, L. W., Kelly, A. L., Wiley, M., Adair, B. M., & Patterson, M. (2007). Inactivation of a calicivirus and enterovirus in shellfish by high pressure. *Innovative Food Science and Emerging Technologies*, *8*, 213–217.

Naidu, A. S. (2002). Activated lactoferrin — a new approach to meat safety. *Food Technology*, *56*(3), 40–45.

Patel, J. R., Bhagwat, A. A., Sanglay, G. C., & Solomon, M. B. (2005). Rapid detection of *Salmonella* from hydrodynamic pressure-treated poultry using molecular beacon real time PCR. *Food Microbiology*, *23*(1), 39–46.

Patel, J. R., Sanglay, G. C., Sharma, M., & Solomon, M. B. (2007). Combining antimicrobials and hydrodynamic pressure for the control of *Listeria monocytogenes* in frankfurters. *Journal of Muscle Foods*, *18*, 1–18.

Patel, J. R., Williams-Campbell, A. M., Liu, M. N., & Solomon, M. B. (2005). Effect of hydrodynamic pressure treatment and cooking on inactivation of *E. coli* O157:H7 in blade tenderized beef steaks. *Journal of Muscle Foods*, *16*, 342–353.

Smiddy, M., Kelly, A. L., Patterson, M. F., & Hill, C. (2006). High pressure-induced inactivation of QB coliphage and c2 phage in oysters and in culture media. *International Journal of Food Microbiology*, 105–119.

Solomon, M. B., Liu, M. N., Patel, J. R., Bowker, B. C., & Sharma, M. (2006). Hydrodynamic pressure processing to improve meat quality and safety. In L. M. L. Nollet, & F. Toldra (Eds.), *Advanced Technologies for Meat Processing* (pp. 219–244). Boca Raton: Taylor and Francis.

Taylor, S., Brock, J., Kruger, C., Berner, T., & Murphy, M. (2004). Safety determination for the use of bovine milk-dervied lactoferrin as a component of an antimicrobial beef carcass spray. *Regulatory Toxicology and Pharmacology*, *39*, 12–24.

Wheeler, C., Vogt, T. M., Armstrong, G. L., Vaughan, G., Weltman, A., Nainan, O., et al. (2005). An outbreak of hepatitis A with green onions. *New England Journal of Medicine*, *353*(9), 890–897.

Williams-Campbell, A. M., & Solomon, M. B. (2002). Reduction of spoilage microorganisms in fresh beef using hydrodynamic pressure processing. *Journal of Food Protection*, *65*, 571–574.